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Studies on the Sulfur-containing Amino Acids and the Related Compounds in Garlic. (I)

Assimilation of Sulfate [^{35}S] in Garlic*

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For the study of sulfate metabolism during the hydroponic cultivation of garlic plant, tracer technique using $^{35}SO_4^{2-}$ was employed. It was found that the roots of garlic possess the high synthetic ability of sulfur containing amino acids and the earliest major labeled amino acids were shown to be cysteine and methionine. After cultivation for 24 hr., the formation of S-allyl-L-cysteine sulfoxide and S-methyl-L-cysteine sulfoxide was observed. In addition to these characteristic sulfur containing amino acids, many unknown sulfur containing amino compounds were detected.

INTRODUCTION

It is well known that garlic (*Allium sativum*) contains at least three characteristic sulfur containing amino acids, S-allyl-L-cysteine sulfoxide (alliin),[1] S-propyl-L-cysteine sulfoxide[2] and S-methyl-L-cysteine sulfoxide,[2] and that these S-alkyl-cysteine sulfoxides are decomposed by the *alliinase* present in garlic. The biosynthesis of these amino acids is not established yet, and there is no literature concerning the biochemical study on sulfur metabolism in garlic plant.

The present paper reports the utilization of inorganic sulfate in growing garlic plants. Using the tracer technique with $^{35}SO_4^{2-}$, the assimilation of ^{35}S in the various periods of cultivation was studied.

EXPERIMENTAL

Material and Method

(1) **Cultivation of plants.** In April, garlic plants (*Allium sativum*, white variety) grown-up to about 30cm. in height were lifted from the ground on the 100th day after sowing. After washing with water, the garlic plants were transplanted into hydroponic bottles (one plant per one bottle), and each of which was filled with 100ml. of hydroponic solution. All feedings were made at the same period. The component of the cultivation solution is shown in Table 1.

(2) **Cultivation of aerial part.** The excised aerial part of garlic plant was cultivated with 50 ml. of hydroponic solution in Table 1 containing 950μC of $H_2{}^{35}SO_4$.

(3) **Incubation of excised roots.** Peeled bulbs of garlic were washed with soap and followed by 70% ethanol. After washing with sterile water, they were soaked for

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Table 1. Component of hydroponic solution.

$Ca(NO_3)_2 \cdot 4H_2O$	0.1 g.
KNO_3	0.025 g.
KH_2PO_4	0.025 g.
$MgCI_2 \cdot 6H_2O$	0.025 g.
$FeCI_3 \cdot 6H_2O$	0.2 mg.
Minor elements*	Trace
$H_2{}^{35}SO_4$ (Carrier free)	4 mC
K_2SO_4	0.05 mg.
Total 100 ml., pH 5.6	

* Arnon A_5 (B, Mn, Zn, Cu, Mo) and Arnon B_6 (V, Cr, Ni, Co, W, Ti)[3] were added.

5 min. in a 0.1% $HgCl_2$ solution and then washed with sterile water. The sterilized bulbs were placed on the moistened filter paper in a petri dish, and kept at 10～15°. After 10 days, the growing roots were cut into pieces of about 2 cm. long. They were collected, washed with sterile water, immediately submerged into 20 ml. of a sterile culture solution in Table 2 and were then incubated at 10～15°.

Table 2. Component of culture solution.*

$MgCl_2 \cdot 6H_2O$	7.2mg.	$MnCl_2 \cdot 4H_2O$	0.09mg.
$Ca(NO_3)_2 \cdot 4H_2O$	4.0mg.	$ZnCl_2$	0.03mg.
NaCl	4.0mg.	H_3BO_3	0.03mg.
KNO_3	1.6mg.	KI	0.015mg.
KCl	1.3mg.	Sucrose	400.0mg.
$NaH_2PO_4 \cdot H_2O$	0.33mg.	Glycine	0.06mg.
$FeCl_3 \cdot 6H_2O$	0.05mg.	Nicotinic Acid	0.01mg.
Pyridoxine HCl	0.002mg.	Thiamine HCl	0.002mg.
$H_2{}^{35}SO_4$ (Carrier free)	1mC		
		Total 20 ml., pH 5.6	

* A modified tissue culture solution of White[4] was used.

(4) Preparation of protein fraction and amino acid fraction. After a fixed period of cutivation, the roots were washed with water. The plants were rapidly cut into roots, bulbs and aerial part, and to prevent the enzymatic reaction each part was placed in a boiling water for 10 min. and after cooling, the materials were homogenized. The homogenate was adjusted with acetic acid to pH 4.0 and centrifuged to separate insoluble protein fraction. The precipitate fraction obtained was rapidly dried at 100° and stored. To obtain the amino acid fraction, the resulting supernatant solution was passed through a column of Amberlite IR-120 (H-type) and the adsorbed amino compounds were eluted with 4% NH_4OH and evaporated to dryness under reduced pressure.

(5) Analysis of sulfur. Sulfur was estimated as $BaSO_4$ according to the method of Pirie.[5]

(6) Determination of radioactivity. Radioactivity was determined with a G-M tube. Samples were oxidized to sulfate according to the method of Pirie, adding

a calculated amount of K_2SO_4 solution as carrier and the sulfate was converted to $BaSO_4$ by the usual manner. They awere counted at an infinite thickness and compared with the count of a standard Ba $^{35}SO_4$.

(7) **Paper chromatography.** Two dimensional ascending paper chromatography was carried out with Toyo No. 50 filter paper 40 × 40 cm. First solvent: PhOH-0.08 % NH_4OH (4 : 1), second solvent: BuOH-AcOH-H_2O (5 : 1 : 4). Paper electrophoresis was also carried out with Toyo No. 50 filter paper 2 × 40 cm. in pyridine-acetic acid-water buffer (10 : 0.4 : 90, PH 6.5) at 30V/cm. The ^{35}S-labeled amino acids were detected by radioautography and by color reaction with nihydrin or iodoplatinate reagent.

(8) **Identification of ^{35}S-labeled amino acids.** The radioactive spots on paper chromatogram were identified by cochromatography with the authentic samples.

RESULTS AND DISCUSSION

(1) **Distribution of ^{35}S in each fractions.** The distribution of ^{35}S in various fractions after the fixed period of cultivation is shown in Table 3. After 30 min. cultivation, the amino acid fraction of roots showed radioactivity and its specific activity as compared with those of bulbs and aerial part according increased more rapidy with an increment of cultivation time. The specific activity of protein fraction was almost similar to that of amino acid fraction. The specific activity of the amino acid fraction of roots was always higher than those of other organs. However, by additional 5 days cultivation without $^{35}SO_4^{2-}$ after 5 days feeding with $^{35}SO_4^{2-}$, almost no more radioactivity could be found in the amino acid fraction of roots, but the specific activity of the amino acid fraction of bulb increased. From these results it is assumed that during additional 5 days cultivation, ^{35}S-labeled amino acids are transported from roots to bulb.

(2) **Assimilation of ^{35}S into excised aerial part and roots.** Excised aerial part and roots were cultivated in a solutlon containing $^{35}SO_4^{2-}$ and the incorporation of ^{35}S into the amino acid fraction was examined. As shown in Table 4, in aerial part, 15% of ^{35}S in cultivation solution was incorporated into the amino acid fraction, however the specific activity of this fraction was much lower than that of roots. From the results of Tables 3 and 4, it is concluded that the S-containing amino acids synthesized both in aerial part and in roots, and that the roots of garlic possess the high biosynthetic ability of sulfur containing amino acids. For the isolation of ^{35}S-labeled amino acids having high specific activity, the authors used the roots of garlic.

Perhaps, sulfur containing amino acids synthetized in both root and aerial part will be transported into bulb, and be stocked there.

(3) **Identification of ^{35}S-labeled amino acids.** ^{35}S-labeled amino acids were identified by the paper chromatography and the autoradiography. The results are given in Fig. 1.

After 30 min. cultivation, cystine and three other unknown compounds were detected as ^{35}S-labeled compounds. After 2 hr. cultivation, methionine was detected. From these results it is assumed that (cysteine) and methionine are synthesized rapidly from

Table 3. Distribution of ^{35}S in amino acid and protein fractions of root, aerial part and bulb of garlic.

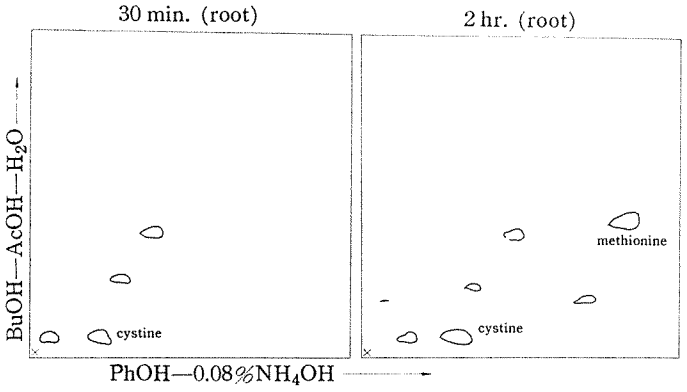
			30min.	2hr.	24hr.	10days*
Root	Weight		12 g.	9	9	8.5
	Total ^{35}S in extract		12,00 μc	37.44	554	33.5
	Amino acid fraction	Weight	75 mg.	60	56	50
		S	5.9 mg.	4.7	4.4	3.9
		^{35}S	3.16 μc	15.16	271.9	0.25
		s.a.**	0.536	3.227	61.818	0.063
	Protein fraction	Weight	0.415g.	0.302	0.431	0.497
		S	4.2 mg.	3.3	3.1	3.0
		^{35}S	0.185μc	0.551	5.726	22.65
		s.a.**	0.044	0.167	1.847	7.550
Aerial Part	Weight		29 g.	31	43	29
	Total ^{35}S in extract		7.08 μc	18.20	122	95
	Amino acid fraction	Weight	142 mg.	160	213	140
		S	15.9 mg.	17.8	23.3	15.5
		^{35}S	0.33 μc	2.72	29.6	14.8
		s.a.**	0.021	0.153	1.272	0.955
	Protein fraction	Weight	1.92 g.	1.830	2.329	1.850
		S	9,8 mg.	10.9	15.0	10.0
		^{35}S	0.078μc	0.251	20.46	42.87
		s.a.**	0.008	0.023	1.364	4.287
Bulb	Weight		21 g.	19	18	25
	Total ^{35}S in extract		8.33 μc	23.60	135	486
	Amino acid fraction	Weight	278 mg.	237	240	318
		S	24.7 mg.	21.2	20.7	29.5
		^{35}S	0.35 μc	3.19	80.3	351.3
		s.a.**	0.014	0.183	3.879	11.908
	Protein fraction	Weight	1.80 g.	1.720	1.49	1.910
		S	5.1 mg.	4.3	4.3	5.8
		^{35}S	0.077μc	0.181	20.46	71.51
		s.a.**	0.015	0.042	4.757	12.329

* After 5 days' feeding with $^{35}SO_4^{2-}$, continuously 5 days cultivation without $^{35}SO_4^{2-}$.
** s.a.: specific activity= μc/Smg.

sulfate in garlic plant. After 24 hr. cultivation, many new radioactive spots were detected. Those spots on the radioautograms of the amino acid fractions of roots were also appeared on the radioautogram of the amino acid fraction of bulb and aerial part. Among the spots, cystine, cysteic acid, methionine, methionine sulfoxide, S-allyl-L-cysteine sulfoxide and S-methyl-L-cysteine sulfoxide could be identified by cochromatography with the authentic samples. After the amino acid fraction was treated with

Table 4. Incorporation of ^{35}S into amino acid fraction of excised aerial part and excised roots.

	Culture solution	Feeding times	Weight g.	Total ^{35}S in extract μc	Amino acid fraction Weight mg.	^{35}S μc	Specific activity μc/mg.
Aerial part	50 ml. in 950 μc $^{35}SO_4^{2-}$	4 days	90	356	640	131	0.2
Roots	20 ml. in 1 mc $^{35}SO_4^{2-}$	44 hr.	2	320	6.5	73.6	11.3



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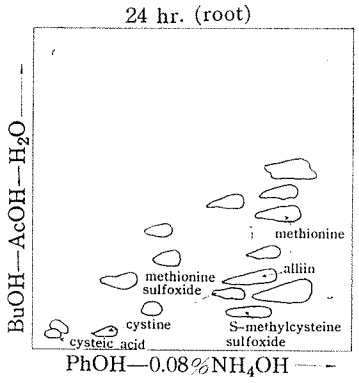

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Fig. 1. Radioautogram of ^{35}S-labeled amino compounds in garlic.

alliinase[1] the spots of the S-allyl-L-cysteine sulfoxide and S-methyl-L-cysteine sulfoxide disappeared.

By 24 hr. cultivation, all kinds of the sulfur containing amino compounds in garlic are synthesized and even by additional 10 days cultivation no more S-containing amino acid appeared.

The identification of the unknown new amino acids detected on the radioautogram will be reported in the next paper.

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